Shin WATANABE*: **The genus *Chlorella* (Chlorococcales) from Japanese soils (2)****

渡辺 信*: 日本産土壌クロレラ属（クロロコッカーレス目）(2)**

Chlorella fusca Shihira et Krauss var. **vacuolata** Shihira et Krauss 1963, 36, fig. 45, 46; Fott et Nováková 1969, 31 pl. 6, 7. (Fig. III)

Adult cells ellipsoidal to spherical; young cells ellipsoidal to subspherical; in old culture most cells spherical. Ellipsoidal cells 2.0−8.0×4.0−10.0 μm in size; spherical cells 13 μm in diam. in old culture; sometimes up to 25 μm in diam. in log-phase in YAW-64. Cell wall thin throughout culture. Chloroplast parietal with many incisions at the margin, or reticulate in some occasions. Pyrenoid spherical, one or rarely two, surrounded with apparently continuous starch grains. Nucleus invisible without stain. Vacuoles abundant, occupying a large portion of cell lumen. Oil droplets present.

Reproduction by 2-8 autospores. Autospores ellipsoidal, with vacuoles, equal or rarely unequal in size in a sporangium. Fragment of mother-cell wall pouch-like.

Plant mass smooth, green, turning to reddish brown in old cultures. Culture No. TOT-61, YAW-64.

Several days after the two isolates had been transferred to newly prepared agar medium, vegetative cells of only YAW-64 were as large as 25 μm in diam. with a large vacuole and a reticulate chloroplast which occupied a small portion of the cell's periphery and appeared saucer-like as a whole. In a one-month-old culture, some individuals revealed the same characters as those in the log-phase, or some reached up to 13 μm in diam. with the chloroplast of the *fusca*-type. At 5 months, however, all of the cells showed the features of normal ones present in the stationary-phase. Sizes of the giant cells in young culture were far different from those of my another isolate and those in the description of previous authors. The reticulate chloroplast of these cells corresponded with the observation of Shihira and

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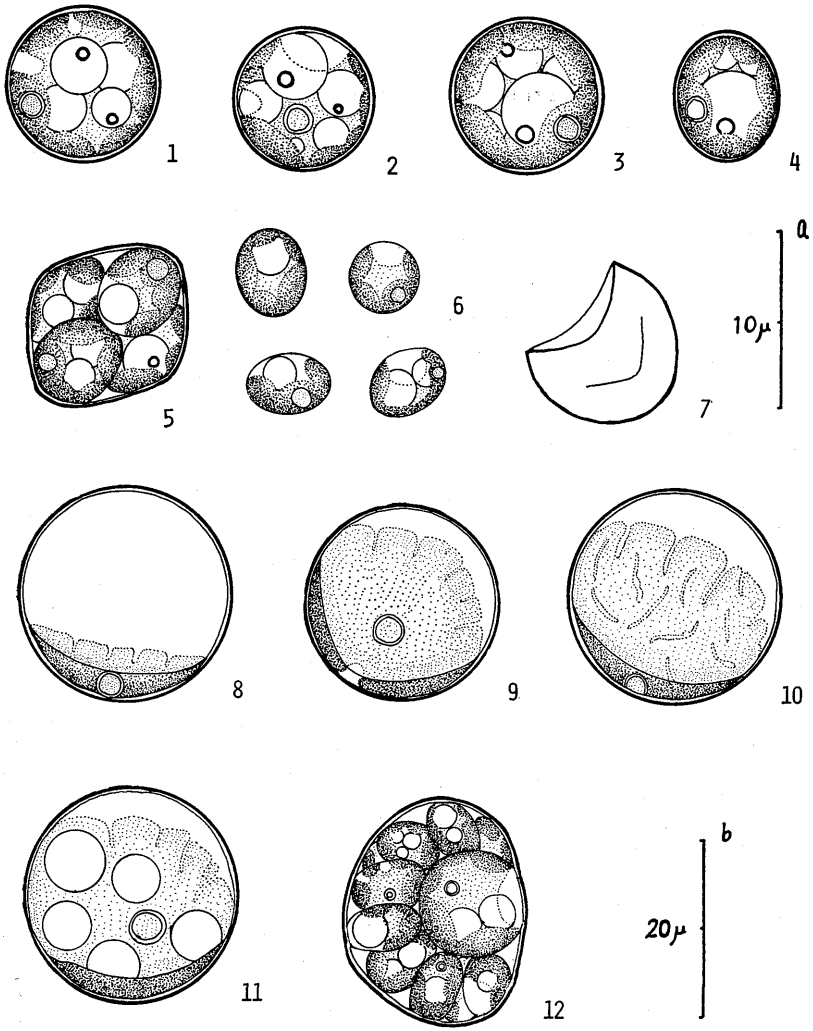


Fig. III. 1-12 : *Chlorella fusca* var. *vacuolata*. 1-6, cells with normal features. 1-3, adult spherical cells with a parietal chloroplast, and with large vacuoles. 4, young ellipsoidal cell. 5, autospores in a sporangium. 6, just liberated autospores. 7, empty mother-cell wall. 8-12, cells with abnormal features. 8 and 9, giant cells with a saucer-shaped chloroplast which is appressed to the cell wall by a large vacuole. 10, giant cell with a torn chloroplast. 11, giant cell with a chloroplast which is forced aside by vacuoles. 12, large irregularly shaped sporangium containing autospores which are unequal in size. Scale *a* for 1-7, and Scale *b* for 8-12.

Krauss (1963). However, Fott and Nováková (1969) noticed that the reticulation of the plastid did not occur in cultures examined by them. This eccentric state of cells was obtained only twice among several transfers of an old clone to a fresh agar medium. Although it was intended to induce these cells in a liquid medium, the isolate simply maintained normal features as those in TOT-61. The cultural conditions for providing giant cells were not determined.

Pseudochlorococcum typicum Archibald (1970) seems identical with var. *vacuolata*. The living strain of *P. typicum* from the Indiana University's Culture Collection, which I studied, revealed the same morphology as illustrations of var. *vacuolata* by Fott and Nováková, who drew them from Pringsheim's strain.

Chlorella saccharophila (Krüger) Migula var. **saccharophila**; Shihira et Krauss 1963, 27, fig. 23, 24; Fott et Nováková 1969, 38, pl. 10.

(Fig. IV, 1-8)

Cells ellipsoidal to ovoid-ellipsoidal, rarely spherical. Cells 2.0-6.0×3.0-8.0 μm in size, rarely 10 μm in diam. in old culture. Chloroplast broadly band-shaped or saucer-shaped, with smooth or undulate margin, occupying up to a half of cell's periphery in young culture and almost entire portion of it in old one. Pyrenoid single, spherical, not covered with starch grains. Starch accumulation lacking. Nucleus invisible without stain. Vacuoles rarely present. Oil droplets present.

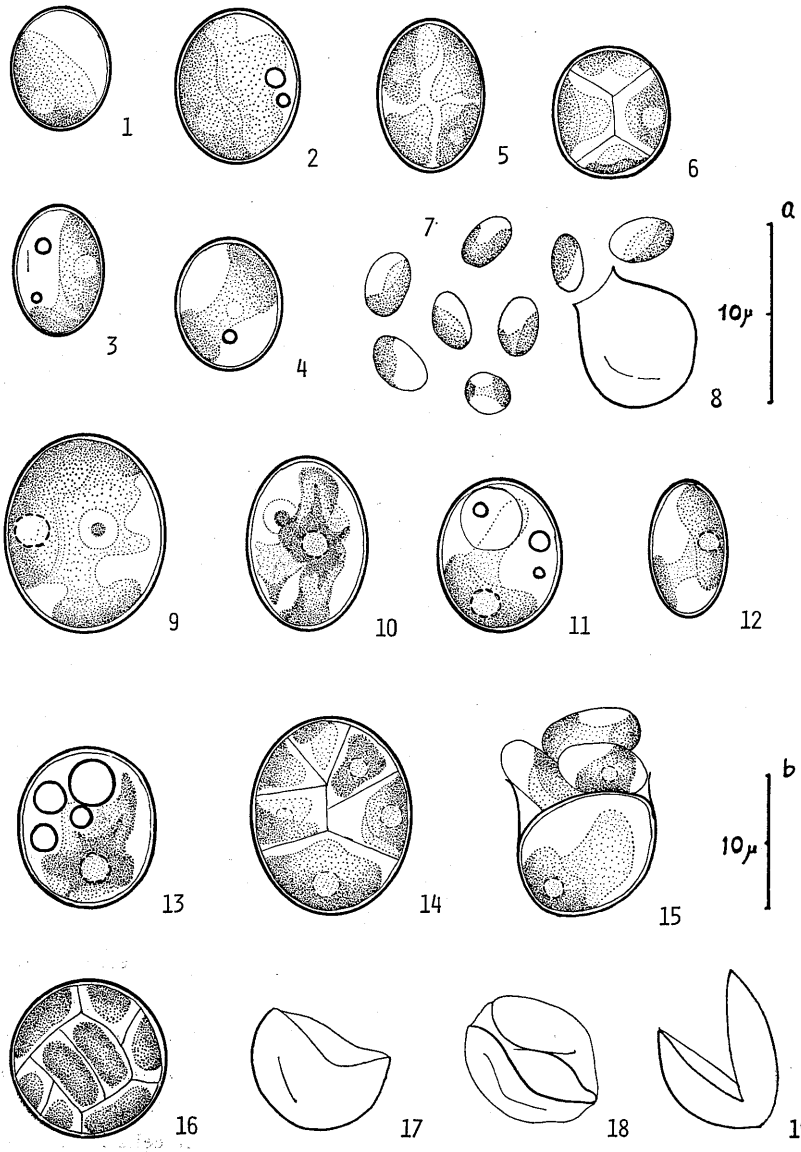
Reproduction by 8-16 autospores. Autospores formed by the progressive cleavage of protoplasm, equal in size in a sporangium. Empty mother-cell wall pouch-like.

Plant mass smooth, green, slightly yellow green in young cultures, turning to relatively dark green in old ones.

Culture No. TOT-44.

Chlorella saccharophila (Krüger) Migula var. **ellipsoidea** (Gerneck) Fott et Nováková 1969, 40, pl. 11, 12. (Fig. IV, 9-15)

Cells ellipsoidal, ovoid-ellipsoidal, ovoid, rarely spherical, 1.5-12.0×2.0-15.0 μm in size or up to 11 μm in diam. Cell wall thin, slightly thickening in old culture. Chloroplast in young cells and autospores band-shaped or saucer-shaped with relatively smooth margin; that in adult cells irregularly undulated, lobed at the margin, frequently detached from cell wall. Pyrenoid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single, spherical, broadly ellipsoidal, nearly in the center of plastid, covered with many small starch grains. A few stroma starch grains sometimes present. Nucleus rarely visible inconspicuously. Vacuoles sometimes present. Oil droplets abundant in old culture.

Reproduction by 4-16 autospores. Autospores formed by the progressive cleavage of protoplasm, unequal in size in a sporangium. Empty mother-cell wall pouch-like.

Plant mass smooth, green, turning to yellow green in old cultures.

Culture No. SAK-20, KIY-3, TOT-62.

Gerneck (1907) described *Chlorella ellipsoidea*, which was later classified as a variety of *C. saccharophila* by Fott and Nováková (1969). Since the diagnosis and explanatory figures by Gerneck were not so complete, the establishment of *C. ellipsoidea* had been questioned by some authors, such as Vischer who summarized the taxonomic problem (1955). Vischer did not adopt the unreliable species of Gerneck and independently described the genus *Jaagia*. The type species of the genus, *J. aquatica*, was morphologically similar to *C. saccharophila* var. *ellipsoidea*. It is possibly presumed that he treated the same species as that of Gerneck and considered it to be a member of a different genus, because he regarded the ellipsoidal cell and the irregular chloroplast detached from the cell wall as striking features enough to distinguish from the genus *Chlorella*. However, such variations in cell shape and chloroplast should be considered to be at the infrageneric rank in these taxonomic groups.

Chlorella reisiglii Watanabe nom. nov. (Fig. IV, 16-19, Fig. V)

Synonym: *Pseudochlorella subsphaerica* Reisigl 1964, 471, fig. 28.

Cells are classed under two types: E-type cells elongate-cylindrical, elongate-ellipsoidal, or elongate-reniform; S-type ones spherical or subspherical.

Fig. IV. 1-8: *Chlorella saccharophila* var. *saccharophila*. 1 and 2, ellipsoidal cell with a saucer-shaped chloroplast. 3 and 4, ellipsoidal cell with a band-shaped chloroplast. 5, cell with divided chloroplasts. 6, cell with dissepiments which enclose divided chloroplasts. 7, just liberated autospores. 8, empty mother-cell wall. Scale *a* for 1-8.

9-15: *Chlorella saccharophila* var. *ellipsoidea*. 9, 11 and 12, ellipsoidal cell with a parietal, band-shaped chloroplast. 10 and 13, ellipsoidal cell with an irregularly shaped chloroplast which is detached from cell wall. 14, cell with dissepiments which divide cell lumen in unequal sizes. 15, autospores in unequal sizes and empty mother-cell wall.

16-19: *Chlorella reisiglii*. 16, S-type autospore formation, with just formed dissepiments; this state indicates an intermediate stage between Fig. V, 2 and 4. 17-19, empty mother-cell walls. Scale *b* for 9-19.

E-type cells swelling into S-type through growth. Adult E-type cells 7.0×10.0 μm in size; adult S-type cells 12.0-18.0 μm in diam.; young E-type cells 2.5 −3.5×5.0−8.0 μm in size; young S-type cells 5.0 μm in diam. at min. Cell wall thin throughout culture. Chloroplasts in young E-type cells band-shaped or saucer-shaped with smooth margin, occupying up to a half of the cell's periphery; those in S-type and in adult E-type cells parietal with undulate, irregularly lobed margin, occupying more than a half of the cell's periphery. Pyrenoids, one or several, ellipsoidal, covered with numerous discontinuous starch grains, locating nearly at the center of chloroplast in E-type cells and at various positions in S-type cells. Stroma starch grains sometimes present. Single, sometimes plural nuclei and nucleoli visible in adult S-type cells. Vacuoles and oil droplets present.

Reproduction by 2-16 autospores. E-type autospores formed more than 8 in number in a sporangium, S-type autospores formed 2 or 4 in it. Mother-cell wall deeply bursts open.

Plant mass smooth, green, not changing in color in old cultures.

Culture No. IWA-14, KIY-15, TOT-1, TOT-41, TOT-42, SAK-1, SAK-35.

The cell sizes of IWA-14, SAK-1, SAK-35 and TOT-42 are similar to those of the original description of Reisigl (1964), while those of TOT-1, TOT-41 and KIY-15 are larger than them. As Reisigl described previously, it appears that two different species are mixed in a single culture because the two types of cells are present. The frequency of occurrence of two types of cells depends on the cultural age. In actively growing culture, young E-type cells dominate over S-type ones. E-type cells are mainly formed when the alga has a high activity to produce autospores, namely the number of them in a sporangium is more than 8. E-type autospores are usually produced in a S-type sporangium and sometimes in an E-type one which can form spores before it swells into S-type. Ordinarily, the frequency of autospore formation in adult E-type cells is much less than that in the S-type. When the growth activity decreases in old culture, young E-type cells swell into S-type and S-type autospores are mainly formed. As a result, S-type cells become more numerous than E-type ones. Although Reisigl mentioned that three-fourth of the total cells were E-type and the remains were S-type, he might have been studying only a younger stage of culture.

Young cell usually possesses a single pyrenoid, and adult S-type cell

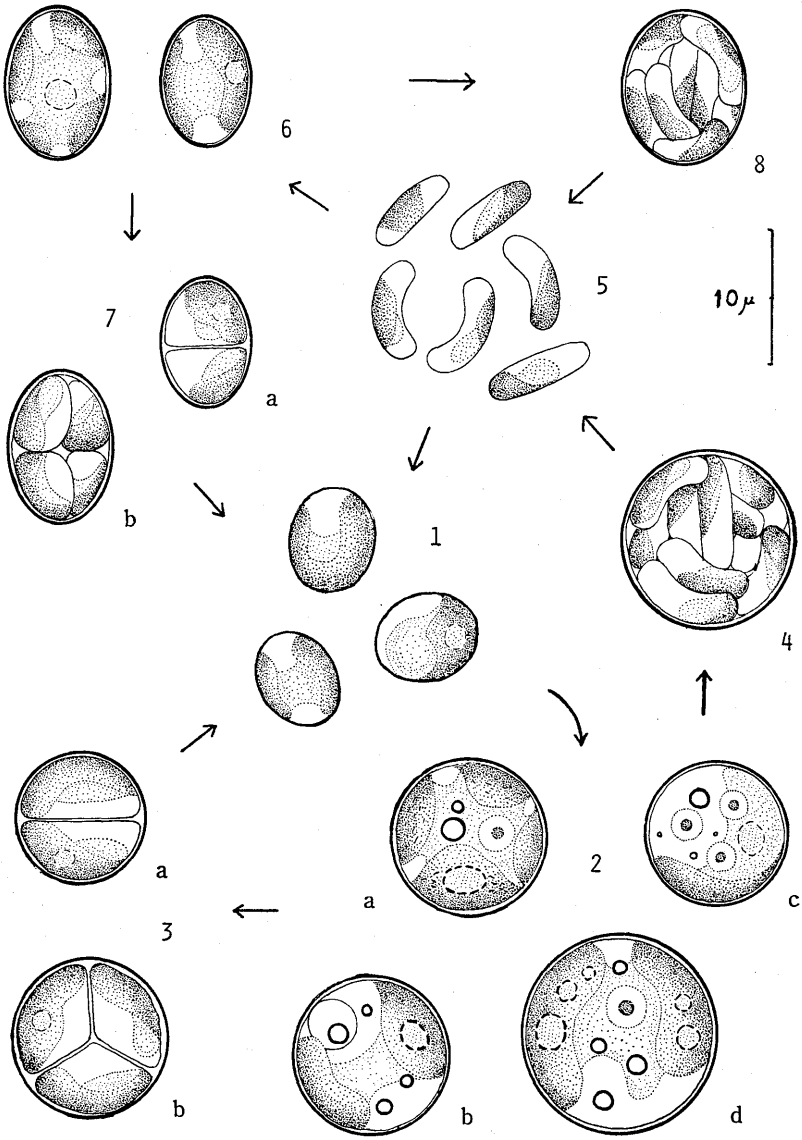

Fig. V. 1-8 : *Chlorella reisiglii*. 1, young S-type cells. 2, adult S-type cells; a, with parietal chloroplast; b, with band-shaped chloroplast; c, with three nuclei; and d, with plural pyrenoids. 3, S-type sporangium containing S-type autospores; a, two autospores; and b, four autospores. 4, S-type sporangium containing E-type autospores. 5, young E-type cells. 6, adult E-type cells. 7, E-type sporangium containing S-type autospores; a, two autospores; and b, four autospores. 8, E-type sporangium containing E-type autospores. Arrows indicate the order of the cell growth.

frequently has 2 or 3 pyrenoids, rarely 9 in number in TOT-41. Since the pyrenoid of this species is multiplied by division, plural pyrenoids result from that the division of the pyrenoid occurs before that of the chloroplast.

Reisigl (1964) emended the diagnosis of the genus *Pseudochlorella* (Zeitler) Lund (1955). According to his characterization, the genus *Pseudochlorella* differs from the genus *Chlorella* in that cells are broadly ovate, irregularly reniform or subspherical. Although these shapes of cells were not described in the diagnosis of the genus *Chlorella*, they seem to be included within this genus, because the variation in shapes of cells in this species is induced by the different activities of autospore formation which change through growing stages. Fott and Nováková (1969) showed that whether autospores are equal or unequal in size in a sporangium is characteristic at the specific rank in the genus *Chlorella*. In the species previously described, autospores are relatively simple in their shapes, even though they are modified to some extent by the irregular formation of dissepiments or by the suppressions in a sporangium. The two types of autospores in this species, however, has a different significance from that proposed by them.

According to the International Botanical Code, *Pseudochlorella subsphaerica* Reisigl has to be emended to *Chlorella reisiglii*, because *C. subsphaerica* is preoccupied by the same author (1964) for the isolate which he did not fully describe, that is, without Latin diagnosis. To avoid confusion, I prefer to call it *C. reisiglii* in honour of Dr. Reisigl who first discovered it from the Alpine region. (To be continued)

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日本各地の土壌から得たクロレラ属の藻株を分離，培養することにより同定した7種2変種のなかの *Chlorella fusca* var. *vacuolata*, *C. saccharophila* var. *saccharophila*, *C. saccharophila* var. *ellipsoidea* の1種2変種が記述された。このほかに *Pseudochlorella* の1種 *P. subsphaerica* Reisigl (1964) が7つの藻株より得られたが，この種はクロレラ属の特徴をそなえていると思われるので属を移動させ，命名規約に従い *Chlorella reisiglii* Watanabe の種名を用意した。